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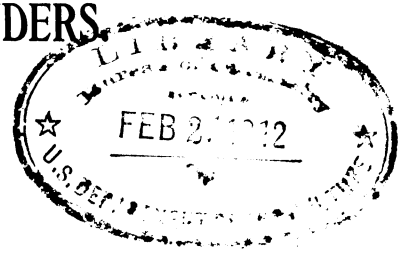
A. D. MELVIN, CHIEF OF BUREAU.

VARIOUS METHODS FOR THE DIAGNOSIS OF GLANDERS.



BY

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Since the discovery of the glanders bacillus in 1883 by Loeffler and Schütz the diagnosis of glanders has been the subject of numerous investigations, and as a result great progress has been made in its determination. The greatest difficulty in the recognition of this disease lies in the fact that many glandered horses do not show positive symptoms until the later stages of the disease. Horses affected with occult or latent glanders and which are apparently not even suspicious cases must be considered as the principal distributing agents of the infection. The early diagnosis of glanders is therefore one of its most important aspects to the practicing veterinarian. With a positive diagnosis definitely established in a stable of horses, subsequent action is clear as to the measures which should be taken to protect the owner from further loss and personal danger.

Our knowledge, methods, and resources in coming to the conclusion that a given horse is or is not affected with glanders has gradually broadened until to-day there are a number of distinct methods by which the diagnosis of glanders may be made, namely—

1. Physical examination.
2. Post-mortem examination.
3. Auto-inoculation.
4. Extirpation of the submaxillary gland.
5. Guinea-pig inoculations.
6. Mallein reaction (subcutaneous, ophthalmo, and cutaneous tests).
7. Serum agglutination reaction.
8. Precipitation reaction.
9. Complement-fixation test.
10. Combined complement-fixation and agglutination test.

PHYSICAL EXAMINATION.

In typical or advanced cases glanders can be definitely diagnosed by physical examination alone. And we are liable not to rely sufficiently on our powers of observation and differentiation in the diagnosis of this disease at the present day when biological tests are so

easily made and laboratories are so much in evidence. The great drawback to physical examination alone is that a large percentage of the cases are not typical, and the so-called occult or latent cases do not show any physical signs. It is nevertheless absolutely essential to pick out these cases if the disease is to be suppressed. It is only too true that the present prevalence of glanders throughout the United States is mainly due to the fact that sufficient effort has not been put forth to get the occult or "contact" animal. We are too likely to content ourselves with the destruction of the more or less marked cases and leave the remaining animals free from quarantine, only to see them break down with the disease at some later date, and in the intervening period to spread the infection to a greater or less degree.

The physical manifestations of glanders are so well known that it is only necessary to lay stress on the most important symptoms to be noted. According to the chief points of localization of the glanders bacilli, we term the disease glanders or farcy. The name glanders is applied when the disease chiefly affects the nostrils and internal organs, while the term farcy is used when the skin is the seat of the disease. It must be understood, however, that glanders and farcy are the same disease, due to the same micro-organism, and vary only in the point of manifestations of the lesions. Farcy appears as swellings of the skin, which vary in size from a quarter to a silver dollar in circumference. They are round, hot, and sensitive to pressure. Soon the center breaks down and the skin sloughs off, leaving a depressed ulcer on the surface which discharges a yellowish serous oily fluid very characteristic to the experienced eye. The lymphatics leading from these ulcers soon become inflamed and stand out under the skin as tense cords, and new farcy "buds" are liable to break out at any point along their course. (See Pl. XXVI, fig. 1.) In glanders the lesions are frequently confined to the respiratory tract and are observable during life on the nasal septum. (See Pl. XXVI, fig. 2.) The lesions here appear first as small, raised nodules about the size of a pea. These soon break down in the center, leaving irregular, lead-colored ulcers, which discharge the same oily tenacious fluid as is seen coming from the farcy buds. As a result a mucopurulent nasal discharge is observed, which may be tinged with blood. In case the ulcers heal they leave irregular scars, which are known as the star-shaped scars of glanders and are quite characteristic. The lymphatic glands under the jaw become enlarged, indurated, and firmly attached to the bone. The above-described nodules and ulcers may occur on the windpipe or in any part of the bronchial tubes. The general condition of the animal becomes poor and the coat staring; otherwise no visible alterations are present, and the animal may live in this condition for years.

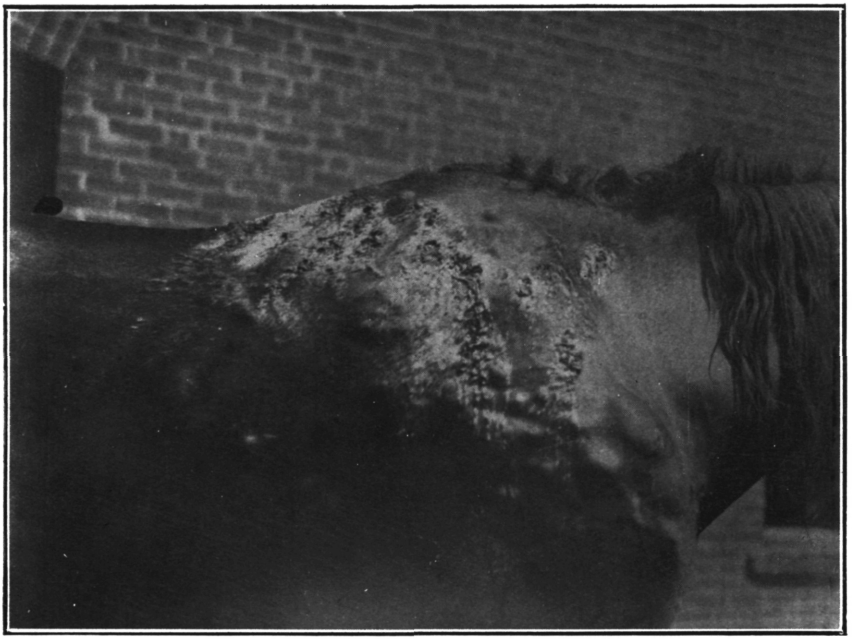


FIG. 1.—FARCY AFFECTING SKIN OF SHOULDER.

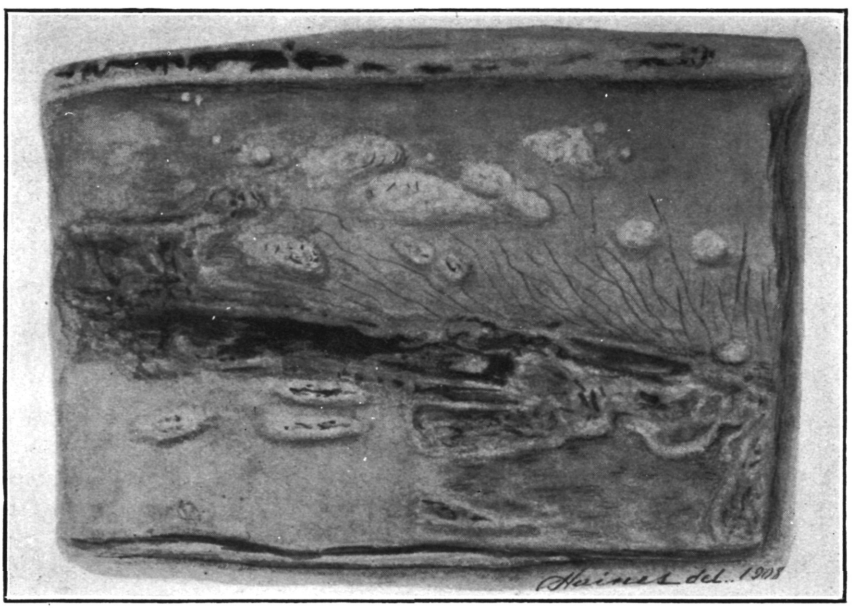


FIG. 2.—LESIONS OF GLANDERS IN NASAL SEPTUM.

In the acute form of the disease the symptoms are the same as those described in the chronic, except that the various stages are more intense and follow each other rapidly. There may be swelling of an entire leg, with numerous farcy buds breaking out, and the nasal septum may be covered with the small nodules and ulcers. The temperature is usually elevated to from 102° to 104° F., the breathing is rapid, and the patient somewhat resembles an animal suffering with pneumonia. The acute form leads to death, and in the chronic cases, which are not destroyed, death usually occurs as a result of the chronic form being accelerated into the acute through hard work or some other condition which reduces the vitality of the patient.

POST-MORTEM EXAMINATION.

This method of diagnosis is of value where a number of animals are showing more or less atypical evidences of glanders and other methods of diagnosis are not available. The animal appearing to be in the most advanced stage should be killed and a post-mortem made. The submaxillary lymph glands should be removed and examined. In cases of nasal glanders these are always involved and are swollen, and in old cases they are bound down to the inferior maxilla by connective tissue. On cross section the parenchyma will be found to contain one or several caseous or purulent centers from a pinhead to a pea in size. The nasal septum should be examined throughout its entire length, as ulceration may be present posteriorly, even though an examination of the anterior portion made during life shows an apparently normal septum. In the early stages of the disease on the nasal septum gray or yellowish nodules of the size of millet seed are present, which are surrounded by reddened mucous membrane. After the breaking down of the nodules ulcers develop, which, as a result of the progressing degeneration, become irregular in shape. (See Pl. XXVI, fig. 2.) Their base is frequently covered with pus and detritus or with a brownish scab. Small ulcers may coalesce, and in this manner large areas of ulceration result. The healed ulcers disclose an almost characteristic cicatrix in the form of a radiating scar, these scars, as before stated, being known as the star-shaped scars of glanders.

The lungs are affected in a large percentage of cases of the disease, and the infection here may be primary or secondary. McFadyean states that no cases of glanders have been recorded in which the lungs were not affected to a greater or less extent. The lungs usually manifest either tubercle-like nodules or lobular pneumonic areas as the initial lesions. The glanders nodule is the most characteristic finding in the lung. These vary in size and appearance in the same animal. They are usually of sizes from a pinhead to that of a pea,

shot-like on palpation, and do not "shell out" readily from the surrounding tissue. In later stages of their development they are found in the center of small hemorrhagic-pneumonic islands. The older nodules are surrounded by firm fibrous capsules. On cross section the center is yellowish, caseous, or dry and crumbling. Calcareous deposits in old arrested glanders nodules may occur. These nodules are usually not in great abundance, and in advanced cases may not number more than 40 or 50 in both lungs. Larger numbers are quite rare.

A catarrhal pneumonic form of glanders of the lungs is manifested in atelectatic areas which have a brownish-red color. In a later stage, however, they break down as a result of a central softening, forming a yellowish caseo-purulent mass, and become surrounded by a red hepatized zone, which is very frequently infiltrated with a yellowish gelatinous exudate. In cases where the lungs show an extensive involvement an acute bronchitis is usually present in association with it. The mucous membranes of the bronchi in such cases show nodules or ulcerations besides the usual characteristics of this affection.

Other organs in which changes occur are the spleen and the liver. These quite frequently manifest small translucent nodules which contain either a yellowish pus or a dry mortar-like mass. The nodules in these organs are usually surrounded by a white dense connective-tissue capsule. Nodules of glanders have also been observed occasionally in the kidneys, brain, body musculature, and in the heart muscle. Of the bones, the ribs are most frequently involved, and in these different sized caverns form, which are filled with a yellowish tenacious substance. The corresponding lymph glands of the affected organs are also usually affected, showing acute swelling in fresh infections, while in chronic cases a dense tough indurated condition is noted.

A differential diagnosis of the nodules becomes very frequently necessary, and in such cases the nodules of anthracosis, metastatic pyemia, malignant tumors, and especially parasitic nodules come into consideration. The nodules of anthracosis are rare. They are small, usually contain pigment, and are composed entirely of fibrous tissue. In metastatic centers of pyemia the pus is more fluid, the connective tissue wall is thin, the condition is acute, and the primary seat of infection can usually be found. In metastatic tumors there are no fibrous walls surrounding the growth, and on cross section the tumor cuts easily and contains no central area of degeneration, but is homogeneous. The primary tumor can be found in some other part of the body. The parasitic nodules caused by the *Strongylus arnfieldi* and the *Ecchinococcus* are quite similar to glanders nodules. Parasitic nodules, however, no matter how small, are always surrounded by a capsule. The periphery is translucent and the center usually con-

tains lime salts. In the large nodules the capsule is not thick as in the old caseous glanders nodules.

Nodular multiplex bronchitis, which is characterized by small, uniform, gray, frequently calcified nodules, may be mistaken for glanders. In the presence of this affection, however, the surrounding lung tissue appears normal; besides, the bronchial lymph glands show no involvement. In chronic interstitial pneumonia the interalveolar connective tissue is extensively and uniformly thickened and there are no nodules and no gelatinous infiltration present. In tuberculosis, which is very rare in horses, there are different sized nodules, some of which resemble sarcomatous growths, and there is also a caseation of the thoracic lymph glands. In botryomycosis and pneumomycosis the changes show a more chronic character, and in these infections a microscopical examination will readily disclose the character of the affection.

AUTO-INOCULATION.

Auto-inoculation consists in a cutaneous vaccination of the suspected horse with its own nasal or lachrymal discharge. It is carried out by shaving the hair and making an incision in the skin, with an ordinary clean scalpel, three-fourths to 1 inch in length and just deep enough to avoid drawing more than a drop of blood. With this same scalpel take up as much of the nasal discharge as will go on its end, and with the flat of the point rub it gently into the incision. The object is to encourage absorption of the virus, which would not be done if there were a large incision and a free flow of blood. The seat of the auto-inoculation must be at a point where it can not be licked or rubbed, and the most convenient point with these qualifications is about 8 inches below and posterior to the ear. In carrying out this procedure on a number of suspected animals, some of which are probably free from glanders, the knife must be thoroughly sterilized after each inoculation, otherwise hetero-inoculation may be produced and lead to very embarrassing results. Between each inoculation, therefore, the knife should be dipped for two or three minutes in 5 per cent carbolic acid solution, removed and wiped with a towel, and passed through the flame of an alcohol lamp. It is better to inoculate all the least-suspected animals first, and then proceed with those more probably diseased.

In the healthy subject, and in those affected but whose nasal discharge contains no glanders bacilli, no untoward result follows the inoculation and the wound heals quickly. In the diseased subject the wound usually heals in the same manner, although rarely a localized glanderous inflammation may occur. This is followed in from one to five days by a rise of temperature of from 1° to 4°, together with the development of various symptoms of glanders. The

auto-inoculation, therefore, in cases of occult glanders stimulates the latent form of the disease into activity and thus allows a diagnosis to be made by physical examination.

There are several objections to this method of diagnosis. The main one is the fact that should it result negatively it does not exclude the presence of glanders, as the nasal discharge does not always contain the specific organism even when the animal is affected with the disease. This may be overcome to a certain degree by repeating the inoculation one or more times. A second objection is the fact that the nasal discharge is very liable to contain other virulent organisms besides the *Bacillus mallei*, which may lead to abscess formation. This, however, has been rare, and Haslan reports only two such cases in a series of 150 inoculations. Should it occur, the abscess can be readily opened, and in the great majority of cases will heal readily. A third objection is the fact that hetero-inoculation may accidentally occur. If the knife be sterilized between each inoculation and the operator use ordinary precaution this danger is hardly to be considered. A fourth objection is the fact that many owners when told of the object of the procedure will not allow it to be done.

This method has not been used in the United States. It has been recommended by several writers, including A. J. Haslan, of the British Army Veterinary Corps, while stationed in India. The method undoubtedly is of value in some cases, and is particularly applicable in such situations as the one in which Dr. Haslan was placed. While he was in India mallein was not issued for use among the army horses, and auto-inoculation was the only means at his command for picking out incipient cases.

EXTIRPATION OF THE SUBMAXILLARY GLAND.

This method of diagnosis was advocated by Haubner, Bollinger, and Dieckerhoff. It consists in the surgical removal of an enlarged submaxillary gland in doubtful cases of glanders and the examination of it bacteriologically for the *Bacillus mallei*. Salmon removed the submaxillary glands from three animals which were killed on account of being affected with glanders. In two of these cases the glands were markedly swollen and contained areas of pus. Bacteriological examination, however, failed to disclose the *Bacillus mallei* in a single case, although all of these animals were proved to have glanders. This method for diagnosis requires a surgical operation and would not be allowed by many horse owners. Furthermore, in case of a negative result the horse can not be said to be free from the disease. This method is therefore considered of no practical value.

DIAGNOSIS BY GUINEA-PIG INOCULATION.

This method was first suggested by Straus, and is often termed "Straus's guinea-pig test for glanders." It is a simple and valuable means of diagnosing those cases of glanders showing farcy buds or a nasal discharge. Although it is usually done at a laboratory, this is not essential, as any veterinarian, with the usual precautions, can apply the test in his office. It is only necessary to have one or two male guinea pigs and an ordinary hypodermic syringe. The nasal discharge is collected on a cotton swab and emulsified with boiled water, so that it can be drawn up into the syringe. The guinea pig is turned over on its back by an assistant, the hair is clipped with scissors from a small area on the abdomen, and the part washed with antiseptic solution. The needle of the syringe is then pushed through the abdominal wall and a few drops of the material injected. The second guinea pig is treated in the same way. Two are inoculated to guard against one dying of peritonitis before the glanders lesions have had time to develop. It is better to allow the animals to live for from three to four days after the inoculation before chloroforming, unless by examining the testicles they are found to be enlarged and the skin covering them hot and reddened, in which case they may be killed at once. The characteristic lesions of glanders are found in the testicle and consist of the thickening of the tunica vaginalis and adhesions between the latter and the testicles. When these are forcibly separated the surface of the testicle is found to contain small white purulent areas. From such a testicle the organisms of glanders can usually be grown in pure culture. Where such changes are found in a guinea pig it is proof that the horse is affected with this disease.

MALLEIN REACTION.

SUBCUTANEOUS TEST.

The first important step toward determining obscure and latent cases of glanders was made by the discovery of mallein. This product was discovered by Kelning, a Russian veterinarian, in 1890. Extensive experiments by Nocard of France and McFadyean of England confirmed the claim of the valuable nature of this diagnostic agent. It is a sterilized filtered extract of the *Bacillus mallei.* The dose of mallein prepared by the Bureau of Animal Industry is 1 cubic centimeter for the average-sized apparently healthy horse. A larger dose, not to exceed 2 cubic centimeters, should be administered to extra heavy, weakened, or aged animals and to those suspected of having glanders. The dose should be reduced accordingly for small animals. Animals exhibiting symptoms of other acute diseases or those with suppurative lesions should not be injected until

they have recovered. The preferable site for injection is on the side of the neck about the center, where any local swelling is plainly visible. The hair should be clipped from an area about 2 inches in diameter, and the skin thoroughly cleansed with a disinfecting solution, such as 5 per cent carbolic acid. Carefully sterilize the syringe and needle before commencing the injection of each group of animals, and immerse the needle in a disinfecting solution before injecting each animal. It is better to use a separate syringe, needle, and thermometer for animals exhibiting symptoms suspicious of glanders. Carbolized oil, vaseline, or lard should be used to facilitate the insertion of thermometers and also to disinfect them. On the day of injection the temperature of each animal should be recorded not less than three times at intervals of not less than two hours; for instance, at 2, 5, and 8 p. m. A careful clinical examination of each animal should also be made, and to each one some designation should be given by which the animal will be known throughout the test. Mallein may then be injected at 8 or 10 p. m., providing the preliminary temperatures are not abnormal. After injection the temperatures should again be recorded, starting at the expiration of not more than 10 hours, and should be repeated at intervals of approximately 2 hours until the expiration of at least 20 hours from the time of injection, and should be continued over a longer period in the case of an animal with a rising temperature at the twentieth hour, if, at the same time, a local reaction is present. What constitutes a reaction sufficient to warrant condemnation of the animal has been the subject of many articles and prolonged discussion. The Bureau of Animal Industry has adopted the following uniform principles for judging the mallein test:

1. In order that a reaction produced by mallein may be considered positive it should evince the characteristics of a typical reaction; that is, a combination of thermal, local, and general reactions.

2. By a typical reaction is to be understood a gradual rising of temperature of at least 3° F. and to above 104° F., the maximum temperature being sustained in the form of a single or double plateau. It should be accompanied by a local as well as a general reaction.

The local reaction consists of an infiltration at the site of injection, forming a large, abrupt, painful swelling, with radiating lymphatics appearing as raised cords, generally attaining greatest prominence at from 18 to 21 hours after injection. The general reaction is exhibited by a stiffened gait, depression, loss of appetite, and accelerated breathing.

3. The presence of a local reaction, especially when associated with a general reaction, should be regarded as evidence of glanders, even if the thermal reaction be slight or absent.

4. Animals giving an atypical reaction and those reaching a maximum temperature of 103° F. should be retested after the expiration of not less than 15 days.

In America the most extensive work with malleination has been done by J. G. Rutherford, of Canada. The Canadian department of agriculture is making successful efforts to eradicate glanders from the Dominion, and since March, 1905, it has adopted the policy of compensation and slaughter of all animals which react to mallein, whether they are showing symptoms of the disease of not. Many veterinarians have advanced the idea that repeated malleination has a curative effect on the disease and that ceased reactors may return to work and be stabled with healthy horses without danger of transmitting the disease. These views have been disproved by Rutherford's observation, as he has traced several outbreaks of glanders directly to these ceased reactors.

There is a considerable proportion of glanderous animals in which mallein fails to give a typical reaction, and, on the contrary, a reaction may follow the injection of mallein in the absence of glanders. Thus, mallein is not an entirely reliable diagnostic agent for determining glanders, nor has it ever been considered as efficacious in the detection of this disease as tuberculin is for the diagnosis of tuberculosis. Judging the mallein test is a procedure in the successful performance of which no hard and fast rules can be laid down for adoption in all instances. In the great majority of cases definite and well-marked results are obtained. There are, however, cases in which, after carefully weighing all the points in the case, we are undetermined what course to pursue. In such cases it is best to quarantine the animals and subject them to a subsequent test by applying the complement-fixation method. Various statistics have been gathered from different sources relative to the reliability of the mallein test in the diagnosis of glanders, and from a study of 6,870 recorded cases mallein has been found to give satisfactory results in 89 per cent of the tests applied.

When mallein gives negative reaction in animals which show no clinical indications of glanders, the test may be considered to be efficient as a rule. The greatest number of errors occur in those apparently healthy animals which give positive reactions but which on post-mortem examination are found to harbor parasitic nodules in the liver or lungs rather than glanders nodules.

Schütz has pointed out, and Olt has confirmed the statement, that on post-mortem examination of many cases of reactors nothing but these parasitic nodules are found, which are in fact quite innocent and very often occur in healthy horses. In the handling of those apparently normal animals it would therefore appear advisable to

consider all nonreactors as healthy, while those which react should be quarantined and subjected to the more accurate complement fixation-agglutination test described on page 364.

OPHTHALMO TEST.

This method of diagnosis has been recommended by various investigators, and variable results have been obtained from this test, but they were not uniformly satisfactory. Nevertheless, Prof. Schnürer,[1] of Vienna, is convinced that with the application of this test by the method recommended by him the disease can be diagnosed in most cases, particularly if in the doubtful cases the agglutination test is employed as an adjunct. The method which is followed in Austria, and which constitutes the official test of that country, is carried out as follows:

The test is made by practitioners. They are furnished with the mallein from the central laboratory at Vienna. Pasteur's "mallein brute" is used, of which 0.75 c. c. are used for 10 horses. The mallein is applied to the eye with a camel's-hair brush in the following way: The eyelids are opened with the index finger and the thumb, as is customary when examining the conjunctiva of the eye. Then the camel's-hair brush, which has been submerged in the mallein, is drawn once forward and again backward over the eye. Only one eye is used, the other serving as a control. Immediately after the application of the mallein to the eye in most of the animals lacrimation, increased reddening, and twinkling of the eye appear; these primary reactions are not specific and disappear in the following few hours. The specific reaction commences as a rule 5 or 6 hours after the application of the test and lasts from 36 to 48 hours, occasionally even longer. It consists in a suppurative conjunctivitis, with reddening, swelling, and suppurative secretions. Of these signs only a suppurative secretion should be taken into consideration. The results are interpreted as follows: (1) The reaction is positive if a suppurative secretion is observed in varying quantities. If the secretion is present only in a small quantity, it is principally visible on the inner canthus of the eye. (2) The reaction is negative in the absence of any secretion. (3) The reaction is doubtful when there is present a slimy secretion or lacrimation after 24 hours.

The judgment should be made not earlier than 12 hours and not later than 24 hours after the application of the test. The examination should be made in a good light. A positive result indicates with certainty the presence of glanders; negative results, however, should not eliminate the possibility of the presence of the disease, and only a

[1] Schnürer, J. Die Diagnose der ansteckenden Tierkrankheiten mittels der neuen Immunitäts Reaktionen. IX. International tierärzt. Kongress im Haag., 1909.

repeated negative test after three weeks excludes suspicion of the disease.

Generally the positive ophthalmo reactions are not accompanied by fever or systemic disturbances. Occasionally, however, affected horses are hypersensitive, so that often a trace of mallein which enters the circulation produces fever. Accordingly, it is advisable to accompany the ophthalmo reaction with temperature measurements. For this purpose the temperature should be taken twice, the first time when the eye test is being made and the second time when it is judged. In a doubtful eye reaction where there is an increasing temperature over 101.5° F., the test should be considered positive if the animal had a normal temperature at the time the test was made.

The following principles should be considered in the application of ophthalmo tests:

1. The test should not be undertaken in the presence of a conjunctival catarrh.

2. The removal of the suppurative secretion (by the stable attendant or by the animals licking each other, etc.) may obliterate the indication of a positive result.

3. Positive reactions may be brought on by irritation of the eye.

4. In very rare cases the ophthalmo reaction runs atypically; it is either abortive—that is, it appears very quickly and disappears in a few hours, or it may appear delayed—that is, after 24 hours; both of these reactions should be considered as doubtful.

5. Very rarely it may occur that both eyes react.

6. Between the intensity of the reaction and the degree of the pathological changes there exists no definite relation.

The experience in this country with the ophthalmo reaction for the diagnosis of glanders is very slight. From the limited number of cases which have been tested by this method it is almost impossible to establish the reliability of the test, and accordingly no recommendations can be made as to its effectiveness. One of the writers personally witnessed the application of this test on a glandered horse in the manner described above, and the inflammatory secretion of the reaction appeared so pronounced in the eye that it could be seen from a distance of 20 yards.

CUTANEOUS TESTS.

Since the application of this method of testing by Von Pirquet in 1907 with tuberculin for the diagnosis of tuberculosis in persons, the cutaneous test, as well as the intradermal test, has also been applied to some extent in the diagnosis of glanders. The results, however, which were obtained from these methods of testing were not sufficient to establish their diagnostic value. The most favorable results

were obtained by Schnürer,[1] who claims some value in these methods of diagnosis. For the cutaneous testing concentrated mallein is used, and the application is carried out by superficial scarification of the skin, after which the concentrated mallein is applied to the scarified surface. The hair is shaved on the side of the neck for a length of about 10 centimeters and a width of 5 centimeters. Then, with the aid of an inoculation vaccination lancet three superficial crisscross scarifications are made in equidistant locations. The form of each scarification is #. The first and the third of these scarified areas is painted over with the mallein, while the middle one serves as a control. The reaction commences 6 hours after the application, continues for 24 hours and then disappears gradually. The extension and thickening of the skin varies in the reacting animals 15–50 by 20–55 centimeters, while the thickening may extend from 1 to 2 centimeters. Of course the middle scarification should show no indication of reaction, but serves as the control.

The intradermal reaction has also been applied, but the results were also more or less unsatisfactory. The edematous swelling which develops as a result of the injection is not always sufficiently characteristic to establish the presence or absence of glanders in an animal. This may be also carried out on the side of the neck, about 0.1 c. c. of the crude mallein being used and injected into the skin proper.

SERUM AGGLUTINATION REACTION.

This is one of the later additions to our resources in diagnosing glanders, and with the improvement herein suggested it promises to prove one of the most valuable adjuncts. It was first suggested by McFadyean in 1896, after this investigator had observed the value of Widal's typhoid fever agglutination test. Later, extensive work was done on this subject by Schütz and Miessner, and their results were published in 1905. They used the dead cultures of *Bacillus mallei*, with the sediment macroscopic method, while McFadyean had applied the hanging-drop microscopic method. Schütz and Miessner's method has been generally adopted, and with the improvement suggested by them the execution of this test is greatly simplified and requires a considerably less time than any other method known for the diagnosis of glanders.

In this country the most extensive trial of diagnosis by agglutination has been conducted by V. A. Moore[2] and his assistants, at Ithaca, N. Y. The method pursued by Moore was based on the technique originally suggested by Schütz and Miessner, but the method as it is now practiced in Germany has many advantages.

[1] Schnürer, J. Die Diagnose der ansteckenden Tierkrankheiten mittels der neuen Immunitäts Reaktionen. IX. International tierärzt. Kongress im Haag., 1909.

[2] Moore, Taylor, and Giltner. The agglutination method for the diagnosis of glanders. American Veterinary Review, vol. 30. 1906.

In using this method of diagnosis it is of primary importance to have a suitable culture of *Bacillus mallei*. All cultures of this organism are not susceptible to the specific glanders agglutins which are present in the serum of glandered horses. When a suitable culture is obtained, which is usually accomplished after testing three or four cultures of the organism from different sources, it is kept in stock in the laboratory, and from it the test fluid is made. When a supply of the test fluid is about to be made the organism is inoculated into the abdominal cavity of a male guinea pig. The guinea pig may be killed as soon as the swelling of the testicles is observed, or may be allowed to die naturally. A culture is then made from the testicles on acid glycerin-agar. This constitutes the first generation of the culture after it has been passed through the guinea pig, and it is essential to have the culture pure and uncontaminated with any other organism. When this culture has made a good growth, which requires about 48 hours, it is transferred to a number of glycerin-agar tubes, or, preferably, to Kolle flasks containing glycerin-agar medium, the number of such inoculations depending upon the quantity of test fluid desired. The preference for the Kolle flasks is given on account of the surface of the medium being much larger than in tubes, and therefore a greater quantity of bacilli can be obtained from them. After inoculating the medium with glanders bacilli, the flasks are placed in the incubator, and after 24 hours it is advisable to allow the condensation water in the culture to run over the surface of the medium. This practice will insure a luxuriant growth of the organism. After another 24 or 28 hours in the incubator the surface of the medium contains usually a good growth of glanders bacilli.

The flasks or tubes are then taken from the incubator and placed in a thermostat, where they are heated for two hours at 60° C. in order to render the bacilli inactive. Then the cultures are washed off with a physiological salt solution, to which 0.5 per cent carbolic acid solution has been added. Fifty to 100 c. c. are used for each flask. The fluid is then filtered through ordinary filter paper.

A sample of the old emulsion, which has been previously titrated and tested, is then taken, and by gradual dilutions of the new test fluid an optical similarity of the two solutions is attempted. The precise optical similarity is established in the following manner: Two beakers are taken, one being marked *O*, the other *N*. They are filled to a height of 2.5 centimeters, the beaker marked *O* with the old and the one marked *N* with the new test fluid. The beakers containing the test fluid are placed in an ice chest for several days. They are then taken out and placed on printed paper, preferably on engraved print, and by looking from above through the fluid the appearance of the printed matter indicates the density of the test

fluids. If it is found that the new test fluid is denser than the old, it is diluted with carbolized salt solution, and this is repeated until there is a uniform density established in the two dilutions. The dilutions should be made very gradually in order not to get the new solution too thin. In that case it can be thickened only by the addition of bacteria.

Three sera of known agglutination power are then taken, and each is tested with both test fluids. In case the agglutination of the new fluid shows the same result as the old one, which is also confirmed by the known agglutination power of the tested sera, then it is proved that the new test fluid is of proper strength. Before these serometric tests are made it is advisable to keep the fluid for several days in the ice chest. These test fluids keep well for about two months at a temperature of from 4° to 6° C.

With the test fluids prepared and titrated, the test can now be undertaken. This is a simple procedure. The blood is taken from the jugular vein of the horse by means of a small trocar and cannula, and the serum is allowed to separate.

The test fluid is distributed into four tubes containing 3 c. c. each. Sufficient serum is then added to make the dilutions 1 to 200, 1 to 500, 1 to 800, and 1 to 1,200, respectively. They are then set aside in the incubator and examined by the old method at the end of 24, 48, and 72 hours for the macroscopic evidence of agglutination.

In the laboratory the serum is diluted with physiological salt solution and measured by means of a finely graduated pipette directly into the test tubes.

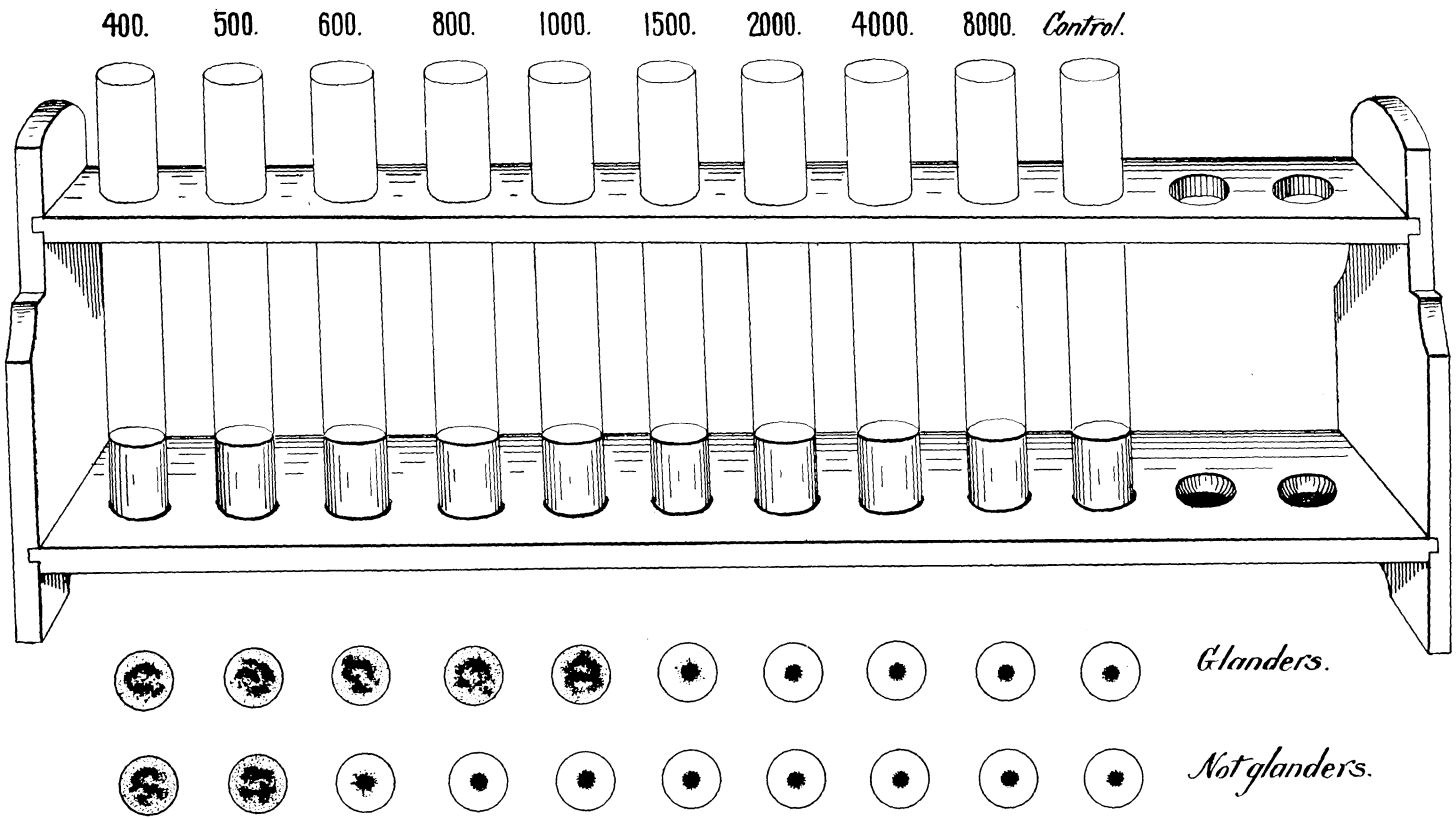
This method of agglutination testing has recently been improved by Miessner,[1] Pfeiler,[2] and Müller,[3] and it assures considerable saving of time in the diagnosis. The preparation of the test fluid as well as the titration is made as described above, while the test proper is executed in the following way:

The pipettes used are of 1 c. c. capacity graduated into hundredths, 12 c. c. pipettes graduated into twentieths, and 25 c. c. pipettes graduated into tenths. Test-tube stands holding 12 tubes with conical shaped holes at the bottom are used. The tubes should be such as may be used for centrifugalization. Nine tubes are placed in the stand marked with the number of dilutions the respective tubes contain. The suspected serum is diluted with carbolized salt solution in the proportion of 1 to 40 (0.5 c c. serum to 19.5 c. c. carbolized salt

[1] Miessner. Die Schnellagglutination und ihre Verwandung bei der Serodiagnose des Rotzes. Centralblatt für Bakteriologie. Abt. 1, Orig., Band 48. 1908.

[2] Pfeiler, W. Über die Serodiagnose der Rotzkrankheit und die Beschleunigung der Agglutination der Rotzbazillen durch Zentrifugieren. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 34. 1908.

[3] Müller, M. Beiträge zur Agglutinationstechnik beim Rotz. Berliner Tierärztliche Wochenschrift. 1908.



TYPES OF AGGLUTINATION IN THE DIAGNOSIS OF GLANDERS.

solution). This constitutes the basic dilution, and from this all the dilutions are made.

From the 1 to 40 basic dilution some of the fluid is drawn up in a pipette graduated into hundredths and the test tubes are successively filled with quantities from this pipette, as indicated by the table below. Then to each tube 2 c. c. of test fluid (bacilli emulsion) are added. The quantity of basic dilution required to make the individual serum dilutions is represented by the following:

Dilution of 1 to 300 equals 0.24 c. c. of basic dilution.
Dilution of 1 to 400 equals 0.2 c. c. of basic dilution.
Dilution of 1 to 500 equals 0.16 c. c. of basic dilution.
Dilution of 1 to 600 equals 0.13 c. c. of basic dilution.
Dilution of 1 to 800 equals 0.1 c. c. of basic dilution.
Dilution of 1 to 1,000 equals 0.08 c. c. of basic dilution.
Dilution of 1 to 1,500 equals 0.06 c. c. of basic dilution.
Dilution of 1 to 2,000 equals 0.04 c. c. of basic dilution.
Dilution of 1 to 4,000 equals 0.02 c. c. of basic dilution.
Dilution of 1 to 8,000 equals 0.01 c. c. of basic dilution.

The tubes are then centrifugalized for 10 minutes in a centrifuge making from 1,500 to 1,600 revolutions a minute. After removal they are allowed to stand for 1½ hours, when the results are read. These results can be perfectly seen by taking the stand containing the tubes and holding it up toward the light of the window to see the bottom of the test tubes. (See Plate XXVII.) The appearance of an irregular, veil-like clumping at the bottom of the tube with a clearing of the upper part of the fluid indicates an agglutination, while the collection of a dense white precipitation at the bottom of the tube and a cloudiness of the upper part of the fluid indicate failure of agglutination.

In carrying out this method of testing in the regular routine of work it is not necessary to employ all of the nine tubes for the test, as four tubes of the dilutions of 1 to 400, 600, 800, and 1,000 are sufficient for ordinary testing. Should the agglutination prove to be higher than 1,000, an additional test to establish the maximum agglutinating power of the serum can be undertaken. By this procedure considerable time can be saved in the execution of the tests.

The serum of most horses free of glanders agglutinates up to the value of 1 to 400, while the serum of glandered horses, as a rule, agglutinates over 1 to 600. On the other hand, experience has proved that horses affected with chronic glanders give occasionally a very low agglutinating value, which in some cases is often lower than that of normal horse blood serum. From this condition it appears evident that in the presence of chronic glanders the disease could be determined in an animal only by repeated tests, and therefore in such cases the diagnosis is possible only from the fluctuation of the agglutination value, as it is a well-known fact that this value is stationary in normal horses.

Sometimes normal horse serum will agglutinate in the value of 1 to 800. However, agglutination hardly occurs above this value in horses free of glanders.

After extensive experience with the agglutination test, Schütz and Miessner[1] established the following rules to serve as guides in judging the results of the agglutination test:

1. All animals suspected of being affected with glanders should be subjected to the agglutination test.
2. All those horses should be destroyed in which the blood shows an agglutination of 1,000 and over.
3. All horses should be destroyed in which the blood shows an agglutinating value of from 500 to 800 and which at the same time show clinical manifestations of glanders.
4. All other horses in which the blood shows an agglutinating value from 500 to 800 should be isolated and destroyed only in case on the second examination the agglutinating value changes.
5. All horses in which the blood shows an agglutinating value of 500 to 800 should be considered free of glanders when the agglutinating value remains unchanged on the second examination.
6. If the presence of the disease has been established in a stable, the blood of all horses should be retested at the end of the third week.

Malleinization of the horses shortly before the blood is taken for the agglutination test influences the results of the test often to a great extent, and therefore it is absolutely essential not to attempt to make the agglutination test in cases where the mallein test has just been made. Ten days to two weeks should elapse before the blood should be taken for the agglutination test from horses which have been tested with mallein.

This method of diagnosis has had considerable trial in the different countries of Europe, and particularly in Austria and Germany. The results obtained proved that the test gave excellent results in acute cases of glanders, and accordingly it serves as a splendid adjunct to the complement-fixation test, which always gives good results in chronic cases.

In this country the agglutination test has been used only to a limited extent, and therefore no bold claims can be made for it. However, its adoption by some of the European governments indicates that it is a valuable means for diagnosing glanders, particularly if adopted as an adjunct to the complement-fixation test.

PRECIPITATION REACTION.

This method of diagnosis was discovered by Kraus and has been given considerable attention in the diagnosis of glanders. It is based upon the fact that when blood serum comes in contact with different forms of extracts of glanders bacilli the precipitins or

[1] Schütz and Miessner. Zur Lerodiagnose der Rotzkrankheit. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 31, 1905.

receptors which are formed in the blood of affected animals from the time the infection first occurs are bound to the bodies in the bacillary extract, producing a precipitation which is manifested by cloudiness at the point of contact of the two fluids.

This method was first applied to glanders by Dediulin in 1900, but the results were not entirely satisfactory. Wladimiroff mixed the serum of glandered and healthy horses with a filtrate of glanders cultures in proportions of 1 to 2 and 1 to 40. The results were not uniform, and his method of application could not be well utilized for diagnostic purposes. The results of Shirnoff were similar. However, he found that by centrifugalizing the mixture the fluid of healthy horses cleared entirely, whereas the fluid containing the serum of glandered horses showed a certain opalescence.

The application of this method, however, appeared to give far more satisfactory results when employed in the manner first described by Pfeiler[1] and Miessner.[2] These two investigators, who evidently worked independently on this method of diagnosis, adopted a technique in which the interfering action of the normal precipitins in the serum was excluded. This is accomplished by applying the solutions in such a manner that a contact layer of the solutions will result.

The method recommended by Pfeiler is somewhat more complicated than the method of Miessner, and it is carried out in the following manner:

About 0.5 c. c. of serum of the suspected horse is drawn off by a pipette and allowed to run down into an Uhlenhuth tube. It is advisable to adopt a method by which the serum is run into the tube at a certain place, preferably on the left side of the tube. By this procedure a road is made by the fluid in the tube. Then the antigen (extract of glanders bacilli) is drawn into a pipette. First, 1 drop is allowed to run into the tube at about the same place where the serum has run down. Then 0.3 c. c. of the extract is allowed to run down on the side of the tube. This procedure is followed in order to insure that the first drop of antigen forms a layer on the surface of the serum, and the antigen added will then collect on top of the fluid without disturbing the point of contact. If a number of tubes are undertaken, all the tubes should receive first the serum, and then by the method indicated above the antigen is added to each tube. The tubes are kept at room temperature, and the results are read not later than one hour. Strongly precipitating sera react in 1 to 10 minutes. The precipitation ring is manifested at the point of con-

[1] Pfeiler, Willy. Die Ermittelung der Rotzkrankheit durch die Präzipitationsmethode. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 35, Heft 4/5, pp. 328–337. June 24, 1909.

[2] Miessner. Die Verwendung der Präzipitation in Form der Schichtungsmethode zur Diagnostik der Rotzkrankheit. Centralblatt für Bakteriologie, 1909, Orig. Band 51.

tact of two solutions in the form of an opaque zone, the density and thickness of which depend on the amount of precipitins present in the examined serum.

The extract (antigen) which is used in the precipitation test is identical with the extract employed in the complement-fixation test. Before the extract is adapted for this testing it is necessary to titrate it. Of the concentrated extract dilutions are made with salt solution in proportions of 1 to 1, 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 8, 1 to 10, 1 to 12, 1 to 15, and 1 to 20. Then 6 sera of non-glandered horses and 6 sera of glandered horses are taken and tested with the various dilutions of the extract. For future testing the dilution of the extract is used which produces the most marked precipitation in the glandered sera and which at the same time gives no precipitation in the sera from horses free of glanders.

The method of Miessner is also carried out in Uhlenhuth tubes in which about 0.5 c. c. of undiluted serum is placed. He employs as antigen mallein which is marketed under the name of "Malleinum siccum Foth." The antigen is carefully allowed to run into the tube in order to have a distinct point of contact develop between the serum and the mallein. He then places the racks containing the tubes into incubators at 37° C. for two hours, after which time the results are read. The reaction is the same as in the method of Pfeiler.

A modification of the precipitation test has been recommended recently by Konew.[1] He prefers the use as an antigen of a fluid which he named "mallease" and which represents a filtrate of glanders bacilli dissolved in antiformin.

This solution of the glanders micro-organism is prepared by dissolving the growth which occurs on a 2-day-old agar culture with an 8 per cent antiformin solution by using about 10 cubic centimeters of the latter to each agar culture. Antiformin is the patented name of a disinfectant made by adding sodium hydrate to a solution of sodium hypochlorid, and is on the market at 60 cents a pint. Its activity seems to be due to an intense oxidation. This solution of antiformin has recently been attracting the attention of those bacteriologists who are interested in sputa examinations on account of its ability to dissolve various forms of bacteria generally found in the sputum without affecting in any way the bacillus of tuberculosis, thereby permitting the latter to be more readily detected on microscopic examination. It has this same ability to dissolve the bacillus mallei, and in two hours at room temperature the washed culture previously referred to is completely dissolved by the solution of antiformin. If the culture dissolves quite rapidly, Konew adds to this

[1] Konew, D. Präzipitationsreaktion als diagnostische Methode beim Rotz. Vorläufige Mitteilung. Centralblatt für Bakteriologie. Abt. 1, Orig., Band 55, Heft 3, pp. 251–253. July 9, 1910.

solution another washed culture of greater density in order to obtain as a final result a saturated or concentrated antiformin solution of glanders bacilli. This solution is at first strongly alkaline, but is neutralized by means of a 5 per cent solution of sulphuric acid. The solution is then filtered, first by ordinary filter paper and later by the Berkefeld filter, in order that the fluid will be homogeneous without any undissolved bacilli being present. This fluid constitutes the one component part of the precipitation reaction, and as a name to distinguish it from the other soluble albumens Konew has termed it "mallease," which is analogous to the terms tuberculase, pyocyanase, etc.

According to its discoverer, the precipitation reaction is carried out in the following manner:

The blood taken from the jugular of the horse to be examined is collected in a glass container and then allowed to remain at room or incubator temperature. The separated serum which is thus obtained serves as the second necessary fluid for the precipitation reaction. In order to produce the reaction 1 cubic centimeter of the mallease is poured into a glass test tube of 3 to 4 millimeters in diameter and 15 centimeters long so that the liquid in the tube is about 3 centimeters in height. Then about the same quantity of the blood serum from the suspected horse is taken in a Pasteur pipette which is introduced into the tube containing the mallease in such a manner that the point of the Pasteur pipette reaches the bottom of the tube. Not until then is the serum allowed to pour very slowly under the mallease. Inasmuch as the serum has a higher specific gravity, it remains on the bottom while the mallease is forced up. The free end of the pipette is then covered with the finger and the pipette is carefully taken out so that the serum is not mixed with the mallease. Such a mixing should also be avoided during the introduction of the pipette into the serum. The two solutions must only come in contact at one point and then the reaction will be very marked.

In case of a positive reaction—that is, when the serum is obtained from a horse affected with glanders—a ring of white cloudiness develops at the point of contact of the two clear solutions, as a result of the precipitin formation, which is particularly marked in good daylight when the tube is placed in front of a window against some dark object. According to the duration of the disease, the white ring develops at various times and in varying intensity. In severe and chronic cases of glanders the serum produces the ring immediately; in slight affections when the lesions are not very marked in the animal, the precipitation reaction appears only in 5 to 15 minutes.

This white cloudy zone is somewhat suggestive of the white ring formed by the presence of albumen in the nitric-acid test of urine. (See Pl. XXVIII, fig. 1.)

Based on his results, Konew drew the following conclusions:

1. By using the concentrated solution of glanders bacilli (mallease), the precipitation reaction can be applied as a diagnostic method even in the earliest stages of glanders.

2. As a result of the simple technique and the short time required for examination (about one hour), the precipitation reaction should be preferred to any other method of diagnosis.

3. Blood from the horses to be examined should be taken before the injection of mallein.

4. The solution of mallease must be titrated in accordance with other standard serums before they are given out in practice, and therefore they should only be prepared in bacteriological laboratories.

COMPLEMENT-FIXATION TEST.

In 1909 Shütz and Schubert[1] published the results of their important work on the application of the method of complement fixation for the diagnosis of glanders. And since their experiments were followed by splendid results, exceeding by far the results obtained from either the mallein or the agglutination test, they recommended that this method of diagnosis in combination with the agglutination test be taken as the official test in Germany. This method, overcoming as it does the disadvantages of the mallein and agglutination tests, constitutes without doubt the most reliable method for the diagnosis of glanders which we have at our command at the present time. The complement-fixation test is, in fact, the most definite method known for determining specific infections and is as nearly perfect as a biological test can be. It has recently been thoroughly studied by this bureau and has given excellent results.[2]

Meyer[3] has also published a recent article on the value of this test in which he concludes that occult glanders may be more readily diagnosed by the complement-agglutination method than by the mallein test.

The principle of this test is presented in the phenomenon of hemolysis, which was first discovered and studied by Bordet and Gengou, and extended by Ehrlich, Morgenroth, and Sachs. It is called the complement-fixation test on account of the fact that the complement has been fixed by the combination of antigen with antibody and thus prevented from participating in the hemolytic process in which it is essential in order for hemolysis to take place. By this method even small quantities of glanders amboceptors (antibodies) can be demonstrated in a serum.

The presence of an infectious principle in the organism of an animal or a man has a stimulating effect on the production of antibodies (immune bodies). If a serum containing such immune bodies is inactivated and brought into contact with the antigen in the presence of complement, the complement will become firmly fixed by the combined immune body and antigen. (See Pl. XXVIII,

[1] Schütz and Schubert. Die Ermittelung der Rotzkrankheit mit Hilfe der Komplementablenkungsmethode. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 35, Heft 1/2, pp. 44–83. 1909.

[2] Mohler, John R., and Eichhorn, Adolph. The Diagnosis of Glanders by Complement Fixation. U. S. Department of Agriculture, Bureau of Animal Industry, Bulletin 136. 1911.

[3] Meyer, Karl F. Sero Diagnosis of Glanders. American Veterinary Review, vol. 39, Nos. 2 and 3. 1911.

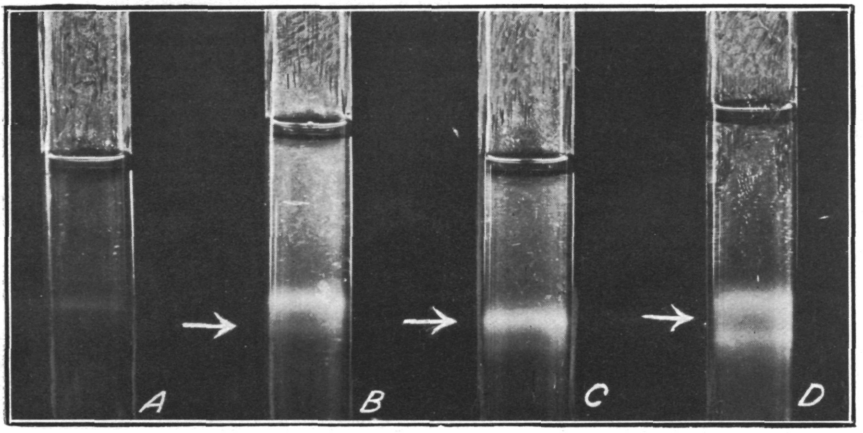


FIG. 1.—PRECIPITATION REACTION FOR GLANDERS.

A. Negative reaction; control test with serum from healthy horse.
B. Positive reaction; serum obtained from an occult case of glanders.
(Note cloudy ring at point of contact of two fluids, indicated by arrow.)
C. Positive reaction; serum obtained from case of nasal glanders.
D. Positive reaction; serum obtained from horse with chronic farcy.

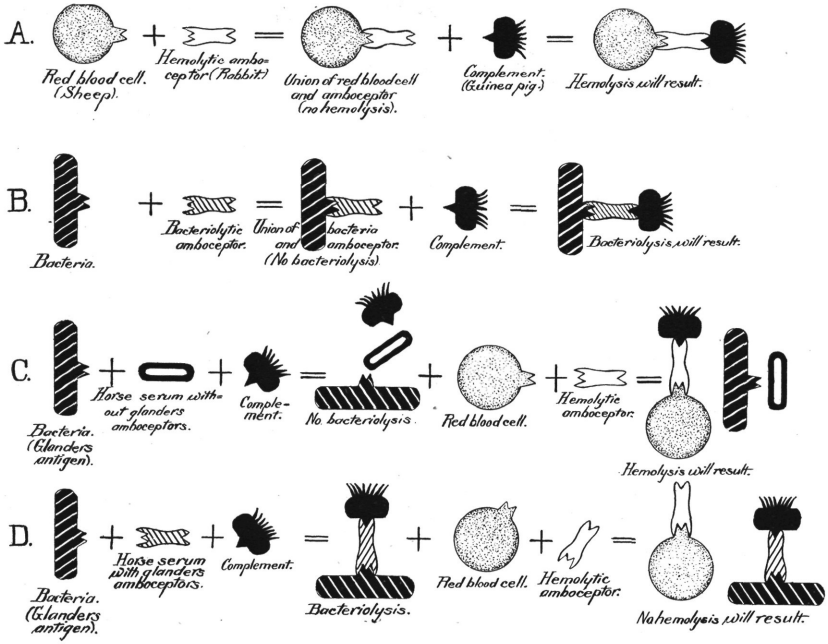
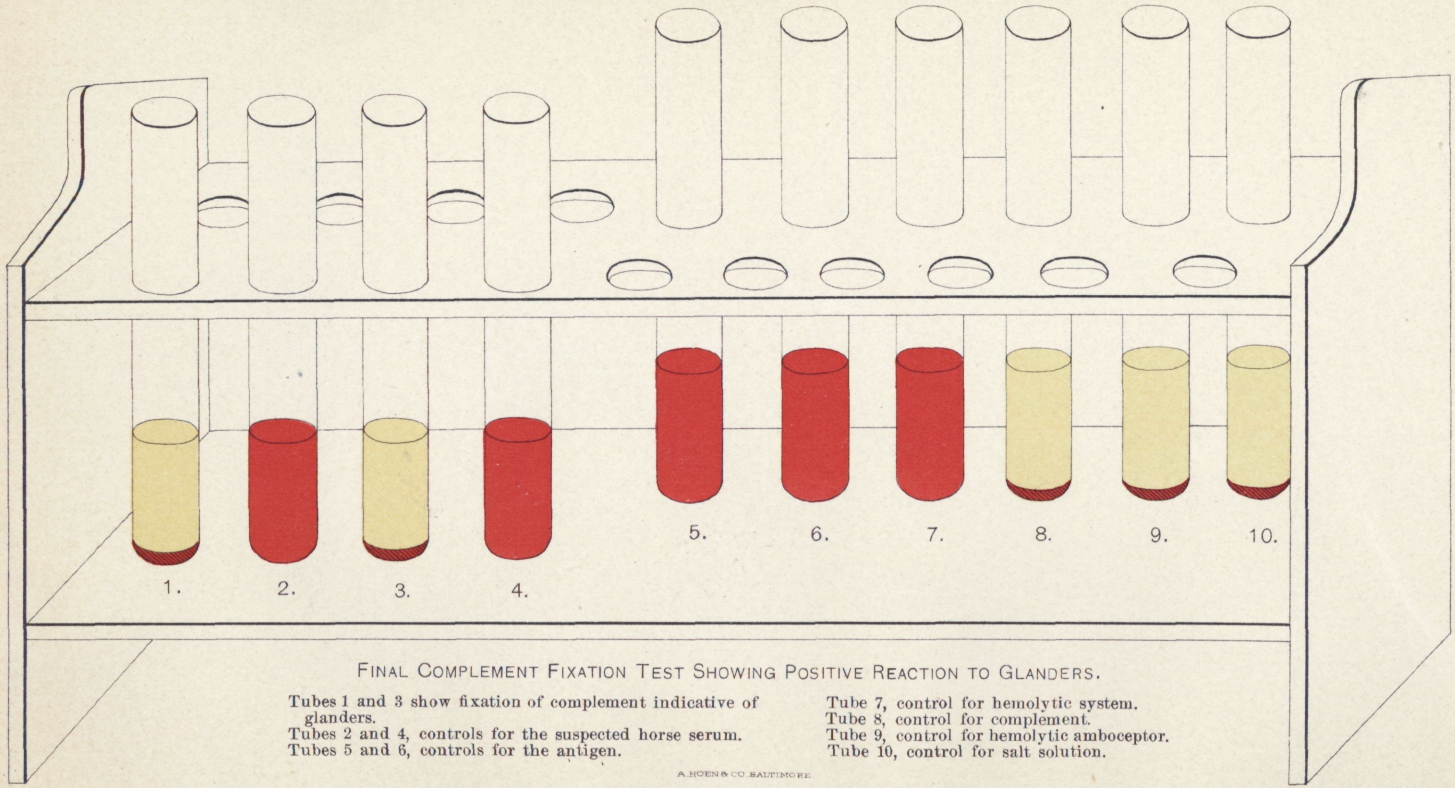


FIG. 2.—DIAGRAMMATIC REPRESENTATION OF COMPLEMENT FIXATION.

A. Hemolytic system.
B. Bacteriolytic system.
C. Negative reaction with normal horse serum.
D. Positive reaction with glandered horse serum.



FINAL COMPLEMENT FIXATION TEST SHOWING POSITIVE REACTION TO GLANDERS.

Tubes 1 and 3 show fixation of complement indicative of glanders.
Tubes 2 and 4, controls for the suspected horse serum.
Tubes 5 and 6, controls for the antigen.
Tube 7, control for hemolytic system.
Tube 8, control for complement.
Tube 9, control for hemolytic amboceptor.
Tube 10, control for salt solution.

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fig. 2, *B.*) Thus, anchoring takes places between the antigen and the antibody in which the complement becomes fixed. This anchoring is thoroughly established when the mixture is placed in an incubator for one hour. The addition of the hemolytic amboceptor and blood corpuscles to such an anchored antigen and immune body will have no effect. (See Pl. XXVIII, fig. 2, *D.*) Thus no hemolysis will take place, inasmuch as the complement has been fixed by the immune body and the antigen, thereby leaving the hemolytic system incomplete. On the other hand, if the inactivated serum contains no immune bodies, there would be no substance in the serum to anchor the antigen. As a result, therefore, no fixation of complement will occur, this being left free, and on addition of hemolytic amboceptor and blood corpuscles hemolysis will now take place. (See Pl. XXVIII, fig. 2, *C.*) Neither the antigen nor the antibody alone can fix the complement and thereby influence hemolysis when the hemolytic amboceptor and blood corpuscles are added. However, in combination the fixation will invariably take place, and on the addition of the hemolytic amboceptor and blood corpuscles hemolysis will not be produced.

Since the discovery of this phenomenon it has been utilized extensively in serum diagnosis, but probably its greatest value has been obtained from the Wassermann reaction for the diagnosis of syphilis. It has also been employed in other diseases with more or less satisfaction, and its great field in bacteriological investigations has not yet been exhausted for the practical diagnosis and determination of immune bodies in serum. In veterinary practice complement fixation is now gradually becoming used for the diagnosis of glanders. This method of diagnosing glanders has given the most favorable results in Germany, and constitutes at the present time the official test for Prussia and other parts of Germany. It has also been used in the diagnosis of other diseases of animals, but not with such success as in glanders.

The presence of the specific immune bodies (bacteriolytic amboceptors) in the serum of glandered horses brings about the fixation of the complement when the antigen in the form of glanders bacilli extract is added to the hemolytic system. The serum of glandered horses, therefore, contains antibodies (immune bodies) against glanders bacilli, which are specific only for the glanders bacilli and for no other infection. The complement fixation accordingly represents a specific test, as only in the presence of the glanderous immune bodies and glanderous antigen will the reaction take place. If, instead of the glanderous immune bodies, other antibodies of another infectious disease be present in the blood serum, they will exert no effect whatsoever on the glanderous antigen; and, on the other hand, if serum containing glanderous immune bodies is brought in contact

with an antigen of another infectious disease it will also have no effect on the reaction. By this fixation of the complement the hemolytic system is left incomplete, and as a result no hemolysis will take place. This fixation of the complement by the antigen and immune bodies of glanders in the horse serum constitutes the diagnostic test for this disease.

In the application of the test it is necessary to have substances constituting the hemolytic system, which are the washed blood corpuscles of a sheep, the hemolytic amboceptor (rabbit serum), and complement (normal guinea pig serum).

There are also used, besides the hemolytic system, the serum of the horse to be examined and antigen (extract of glanders bacilli). The preparation of these various substances and the technique of their application is explicitly presented in Bureau of Animal Industry Bulletin 136, previously mentioned.

The results of the tests are manifested in most instances by a distinct reaction which takes place in the test tubes.

We may thus obtain in these tubes either complete hemolysis, incomplete hemolysis, or no hemolysis whatsoever. The fixation of the complement is manifested by the absence of hemolysis, and therefore we have a settling of the blood corpuscles with the watery clear fluid above. Such a result indicates without doubt the presence of glanders. (See Pl. XXIX.) On the other hand, if the tubes show complete hemolysis, the absence of glanders is thereby indicated. In the presence of glanders a fixation of the complement takes place, as a result of anchoring to the immune bodies and antigen (see Pl. XXVIII, fig. 2, *D*), while in the absence of glanders, there being no immune bodies present, the complement is used up in the phenomenon of hemolysis. (See Pl. XXVIII, fig. 2, *C*.)

Then, again, we may have cases in which the fixation of the complement is incomplete; that is, there is a settling of corpuscles in the bottom of the test tube, but the fluid shows traces of hemolysis. It does not show the characteristic saturated color of hemolysis, but only a tingeing with the hemoglobin. This is termed an almost complete fixation, and also indicates the presence of glanders. The presence of the characteristic color in the fluid and a very slight deposit of corpuscles on the bottom should not be taken as an indication of an infection, as such a condition may be brought on by various causes, and particularly so by the presence of nonspecific substances in the serum of the horse, which may cause a very slight checking of the hemolysis. But all cases where the results show a fixation of the complement (no hemolysis) or almost complete fixation (slight tingeing of the fluid above the settled blood corpuscles) indicate the presence of glanders.

The results of the complement-fixation test should be interpreted as follows:

1. Horses in which the serum produces a complete fixation of the complement in the quantities of 0.1 and 0.2 c. c. should be considered as glandered.

2. Horses in which the serum gives a complete fixation in the quantity of 0.2 c. c. and an incomplete fixation in the quantity of 0.1 c. c. should likewise be considered as glandered.

3. Horses in which the serum produces an incomplete fixation of the complement in the quantities of 0.1 and 0.2 c. c. should also be considered as glandered.

4. Horses in which the serum shows no fixation of the complement in either tube should be considered free of glanders.

In order to reduce the possibility of error to a minimum the agglutination test may be applied to the latter cases, and if this shows a value of 1 to 1,000 or over, the animal should be considered as glandered. However, such cases are extremely rare.

Since this method of diagnosis for glanders was inaugurated in this laboratory large numbers of horses and mules have been examined in the District of Columbia, as well as the blood of animals from other parts of the United States. Many of the horses examined had clinical cases of glanders, while others were selected because they were reactors to the mallein test, some typically, and others atypically. A large proportion of the cases, however, were exposed or "contact" horses. From the number of tests already made—about 1,540—the results indicate that in the complement fixation we have a method which in accuracy is equal to the tuberculin test for the diagnosis of tuberculosis in cattle. The results of the tests thus far conducted show that at least 97 per cent of the cases of glanderous affections can be determined by the complement-fixation method. Furthermore, the affected horses in which a negative or an atypical reaction occurs are as a rule either very old chronic cases of glanders, or those fresh cases of infection tested during the period of incubation. According to Hutyra and Marek [1] the diagnosis of glanders by the complement-fixation test has already given such accurate results that it may be considered as the best method for the determination of this disease at the present time.

Among the horses tested by complement fixation there were a number of animals which gave an atypical reaction to the mallein test, but on the complement-fixation test proved either absolutely positive or negative. Of these horses those which gave a positive reaction and were killed proved to be glandered. The table following shows the comparative results obtained with the mallein and complement-fixation tests.

[1] Spezielle Pathologie und Therapie der Haustiere. Third edition, Band I, p. 717, 1910.

Comparative results with mallein and complement-fixation tests.

Locality.	Positive to mallein test.					Negative to mallein test.			Atypical reaction to mallein test.					Total animals.
	Total number of tests.	Response to complement-fixation test.		Post-mortem.		Total number of tests.	Response to complement-fixation test.		Total number of tests.	Response to complement-fixation. test.		Post-mortem.		
		Positive.	Negative.	Positive.	Negative.		Positive.	Negative.		Positive.	Negative.	Positive.	Negative.	
California	6	4	2											6
Canada	1		1											1
Connecticut	9	8	1	3					1	1		1		10
Florida	9	5	4			9	8	1	6		6			24
Illinois	6	4	2						4	4				10
Indiana	5	3	2			11	2	9	3	1	2	1		19
Maine	7		7		7	32		32	12		12		3	51
Michigan	18	17	1			3		3	6		6			27
Missouri	1	1							9	3	6			10
Montana	35	26	9			17	4	13	8	4	4			60
Nebraska	6	5	1						6	3	3			12
North Dakota	19	19				11	11		12	6	6			42
Oregon	2	2							1	1				3
Pennsylvania	1		1						1		1			2
Texas	5	2	3						4		4			9
Washington	2	2							1	1				3
Wyoming	5	5							2		2			7
Miscellaneous									29	20	9	20		29
Total	137	103	34	3	7	83	25	58	105	44	61	22	3	325

In addition to the above there have been 1,218 tests made upon horses with the complement-fixation method alone, and we have also tested by this method the blood of one lion, which gave a positive reaction, and the blood of one human suspected of having glanders, which proved negative. These two results were later substantiated.

Of the above-mentioned 1,218 tests, 643 were conducted on horses at Washington, D. C., and of these 21 gave a positive reaction, all of which were subsequently confirmed on post-mortem. The remaining 575 were from miscellaneous sources, 78 of which were positive, while 497 were negative.

In the 325 cases shown in the above table, wherein the two tests are compared, the mallein test was confirmed by the complement-fixation test in 161 cases and was not confirmed in 59 cases. There were 105 atypical reactions to mallein which were definitely diagnosed by complement fixation—44 positive and 61 negative. Seven of the Maine reactors to mallein and 3 atypical reactors were examined post-mortem without showing any evidence of glanders.

In order to determine whether the fixation of the complement may be obtained occasionally in normal horses or in horses affected with various diseases other than glanders, a number of tests were made with the blood of apparently normal horses, and also with horses suffering with various infectious and noninfectious diseases. One of these tests was made with the blood of a horse affected with swamp fever, in which the temperature registered 106.2° F.; other tests were

made with blood from horses affected with distemper, influenza, pneumonia, heaves, lameness, fistulous withers, forage poisoning, etc., but in all these cases negative reactions were obtained.

COMBINED COMPLEMENT-FIXATION AND AGGLUTINATION TESTS.

While the complement-fixation test is without doubt the most satisfactory single method of diagnosing glanders, and although practically all cases giving a complement fixation can be considered as glanderous infections, nevertheless there is a very small percentage of cases in which the complement fixation is not well marked, and in these instances some uncertainty may be felt regarding the final determination of the presence or absence of the disease. Such instances may be met particularly where the infection is of a very recent origin. Inasmuch as it has been established that the agglutination test gives highly satisfactory results in these early stages of glanders, the application of the combined test appears therefore very advisable. The results which were published by Miessner and Trapp [1] regarding the value of the complement-fixation test and the combined blood test (complement fixation and agglutination) show that about 97 per cent of the cases in which complement fixation is obtained prove to be glanders. On the other hand, by the combined blood test the number of failures in healthy horses is reduced to 1.1 per cent, and in glandered horses to 0; or, as Hutyra and Marek [2] have stated, the combined blood test will prove accurate in 99 per cent of the tests applied.

The agglutination test as it is employed in combination with the complement-fixation test is a modification of the agglutination test which was formerly used, and has been described in detail under the heading of "serum agglutination reaction," on page 356.

Based on the experience gained with the combined blood test, the Prussian minister of agriculture has adopted the following principles for the diagnosis of glanders by this method:

1. Horses the serum of which produces a complete diversion of the complement in the quantity of 0.1 c. c. should be considered glandered without consideration of the agglutination value.

2. Horses the serum of which gives an incomplete diversion of complement in the quantity of 0.1 c. c. or even in the quantity of 0.2 c. c. should be destroyed without consideration of the agglutination value.

3. Horses the serum of which produces no diversion of complement in quantities of 0.2 c. c. should be destroyed if their agglutination value exceeds 1 to 1,000.

4. In every stable of horses where the first blood examination reveals glanders, a second series of samples should be taken on the day of the killing of

[1] Miessner and Trapp. Die Komplementbindung beim Rotz und ihre Beziehung zur Syphilisreaction. Centralblatt für Bakteriologie. Band 52. 1909.

[2] Ibid., p. 716.

the affected animals. If glanders is again found, a third series of samples is taken 14 days after the second series and following the disinfection of the premises. Should the third blood examination prove the presence of additional cases of glanders, the procedure should be repeated, as after the first blood examination.

5. Horses the serum of which does not produce a diversion of complement in quantities of 0.2 c. c. and the agglutination value is less than 1,000, should be considered healthy if the blood was taken at least 14 days after the removal of the sources of infection. If the time when the sources of infection were removed can not be positively determined, a second series of blood samples should follow the first. If the second examination of the blood shows the same result as the first, the horses should be considered healthy.

6. The blood examination of the horses in the stable should be considered as concluded when the above requirements have been carried out.